Arch. Biol. Sci., Belgrade, 60 (2), 163-168, 2008 DOI:10.2298/ABS0802163O

EFFECT OF PROTAMINE SULFATE ON THE ISOLATED MESENTERIC ARTERIES OF NORMOTENSIVE AND SPONTANEOUSLY HYPERTENSIVE RATS

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Abstract — We tested the relaxant effect of increased protamine sulfate (PS) amounts (10, 20, 50, 100 and 150 μg/ml) on the isolated mesenteric arteries of normotensive and spontaneously hypertensive (SH) rats, with or without endothelium. PS caused concentration-dependent relaxation of isolated mesenteric arteries in both types of rats. The relaxation effect of PS was lower in SH rats than in normotensive ones. Our results indicate that the vascular smooth muscles play a significant role in PS-mediated relaxation.

Key words: Protamine sulfate, mesenteric artery, hypertension, vascular endothelium, rats

UDC 616.12-008.331.4:59

INTRODUCTION

Protamine sulfate (PS) is a polycationic amine used clinically to reverse heparin overdose (Pugsley et al., 2002), but its function is not yet fully understood (Olive, 2006). The administration of PS to patients who received heparin during cardiopulmonary bypass (CPB) induces hypotension. Four experimental protocols from the Mayo Clinic, Rochester, MN, dealing with the intrinsic mechanism of PS vasodilatation suggested the important role of endothelium and the endothelium-derived relaxing factor (EDRF) nitric oxide (NO) (Viaro et al., 2002).

Although PS is related to histamine, the mechanism of protamine-induced hypotension is not histamine-related (Behne et al., 1994). Therefore, general prophylaxis using H1/H2 receptor antagonists does not seem to be justified and cannot be recommended. PS has been found to have an endothelium-dependent relaxing effect on isolated renal arteries (Oreščanin et al., 2003). These results indicated a better relaxant effect of PS on the isolated renal artery of normotensive rats compared to spontaneously hypertensive (SH) animals in the sense of better preservation of endothelium in normotensive animals. The relaxant effect of PS occurs due to NO release with artery conductance, which is not the case with microvessels, where this effect occurs due to endothelium-derived hyperpolarizing factor release (Cable et al., 1999). Multiple mechanisms of PS action also include inhibition of the carboxypeptidase N-mediated degradation of bradykinin, a peptide that causes vasodilatation and tissue-type plasminogen activator (t-PA) release. Increased bradykinin contributes to protamine-related hypotension through its B(2) receptor in ACE inhibitor-treated patients (Pretorius et al., 2005). Some studies suggest that the cardiovascular depressant actions of PS result from a direct effect on the heart and that PS may produce aberrant conduction within the heart which may result in deleterious effects in heart function, especially conditions associated with myocardial disease (Pugsley et al., 2002).

The aim of this experiment was to study the relaxing effect of PS on the isolated mesenteric arteries of normotensive and SH rats and determine

the role of endothelium and vascular smooth muscle in these reactions.

MATERIAL AND METHODS

Artery preparations

Experiments were performed using mesenteric arteries isolated from normotensive and SH male Wistar rats (250-300 g, 6 months old). All protocols for handling the rats were approved by the local Ethical Committee for animal experimentation, which strictly follows international regulations. There were four experimental groups: isolated mesenteric arteries with (E^+) and without (E^-) endothelium from normotensive and SH rats. The adhering perivascular tissue was carefully removed from arteries cut into 3-5 mm ring segments and incubated for 30 min in Krebs-Ringer bicarbonate solution at 36°C continuously oxygenated with a gas mixture (95% oxygen and 5% carbon dioxide). The rings were equilibrated for 30 min under 2 g of resting tension. An isometric transducer (Ugo Basile, Comerio, Italy) registered mechanical contractions. Contractions of isolated blood vessels were provoked by phenylephrine (10^{-6} M) (Sigma-Aldrich, Taufkirchen, Germany) and the functional integrity of the endothelium was confirmed with acetylcholine (10^{-5} M) (Serva Feinbiochemica, Heidelberg, Germany) and by histopathological examination (Milovanović et al., 2004). The percentage of relaxation caused by acetylcholine depended on the degree of endothelial preservation. In SH rats the endothelium is continuously damaged due to high blood pressure, and the relaxation effect of acetylcholine is therefore much lower than in normotensive rats.

In our experiment, the effects of increasing concentrations of PS (μg/ml: 10, 20, 50, 100, 150) were studied on mesenteric arteries precontracted by phenylephrine (10^{-6} mol) from normotensive and SH rats.

Statistical analysis

The main effects were tested by three-way ANOVA with the rat category (normotensive and SH), the absence or presence of endothelium (E^- and E^+), and PS as factors. The data were post hoc compared using Tukey's standardized test. Newman-Kelus' test was used for comparisons between different concentrations and multiple dose-response curves.

RESULTS

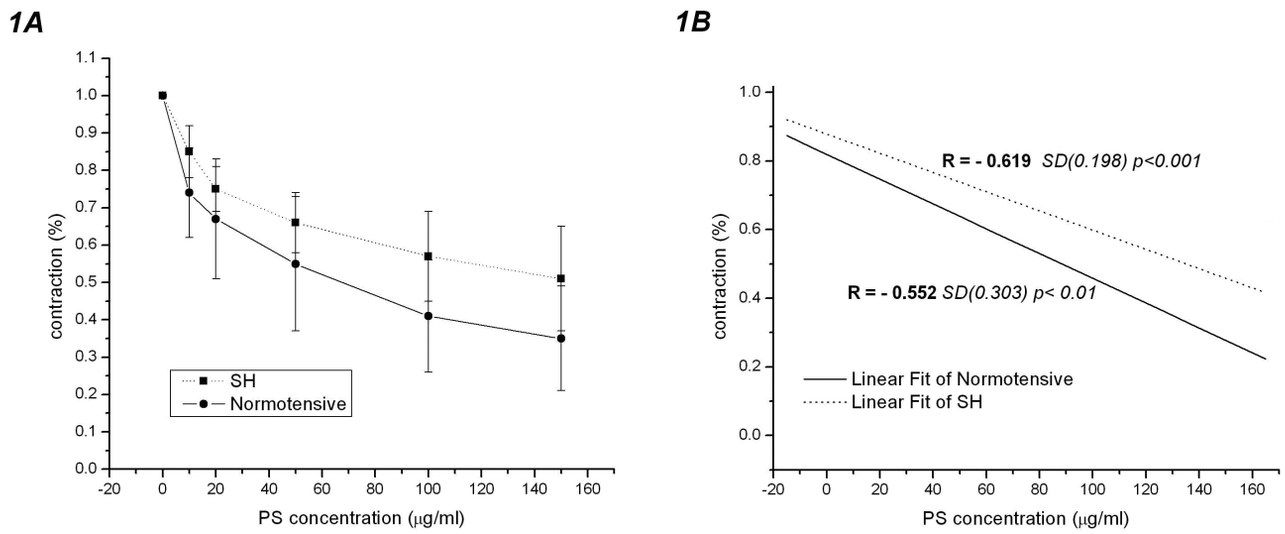
PS caused concentration-dependent relaxation of isolated mesenteric arteries (three-way ANOVA, Table 1). Multiple dose-response curves showed a concentration-dependent PS effect, with a statistically significant high regression factor (Graphs 1B and 2B). However, lower concentrations (10 and 20 μg) showed a greater degree of relaxation than the other concentrations applied ($p<0.001$, Tukey's post hoc comparison of data). The PS-mediated relaxation was greater ($p<0.05$) in mesenteric arterial rings isolated from normotensive rats than in those from SH animals (Table 1, Graphs 1A and 2A).

DISCUSSION

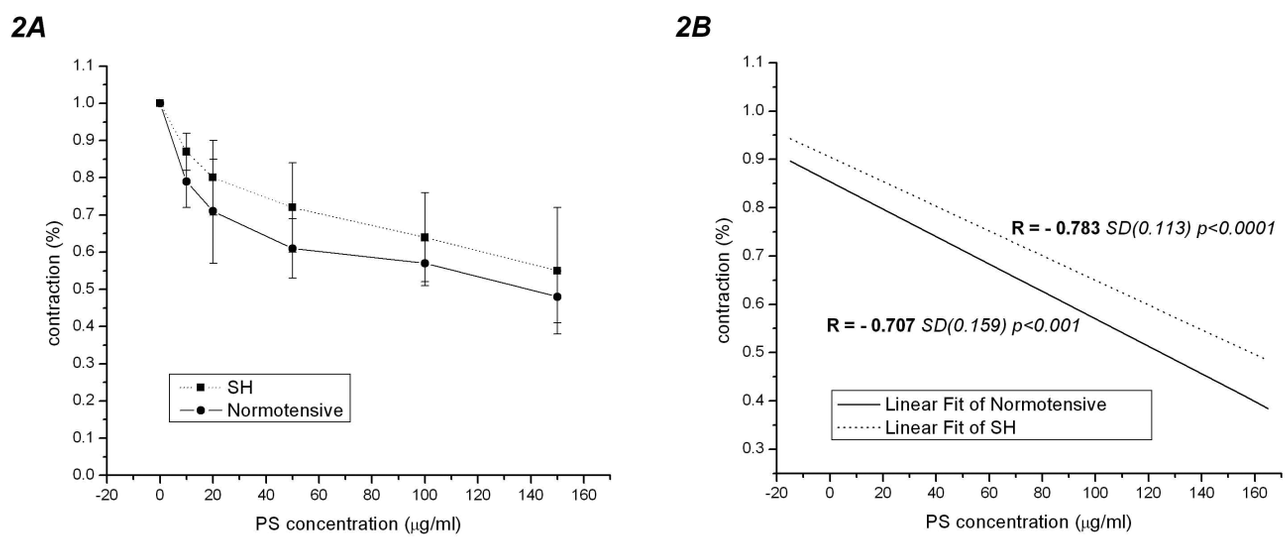
Our results show concentration dependent relaxation of isolated mesenteric arteries of normotensive and SH rats caused by PS. The relaxation effect was better in normotensive than SH rats. Although there was no statistical significance for endothelium-dependent PS relaxation, the main difference between normotensive and SH rats is impaired endothelium function in the latter. This indicates the significance of vascular endothelium in the process. Vascular endothelium has a significant role in hypertension. It is an important metabolic and endocrine organ with a great role in

Table 1. Three-way ANOVA for PS effects. Factors were: the type of rats (R), the presence of endothelium (E), and the presence of protamine sulfate (PS). DF – degrees of freedom, MS – mean squares, F – factor.

Effect	*DF*	*MS*	*F*	*p*
R	1	0.221	5.07	***P<0.05***
E	1	0.087	1.99	N.S.
PS	5	0.686	15.77	***P<0.001***
R x E	1	0.007	0.15	N.S.
R x PS	5	0.010	0.22	N.S.
E x PS	5	0.08	0.18	N.S.
R x E x PS	5	0.002	0.05	N.S.
Error	96	0.047		



Graph 1A. Dose-response curves for relaxation of mesenteric arteries of normotensive and SH rats with endothelium (E^+) induced by increasing concentrations of PS (10, 20, 50, 100, and 150 μg/ml). Data are expressed as means ± SE (the number of observations = 5). 1B. Regression lines (data sets as presented in Graph 1A)



Graph 2A. Dose-response curves for relaxation of mesenteric arteries of normotensive and SH rats without endothelium (E^-) induced by increasing concentrations of PS (10, 20, 50, 100, and 150 μg/ml). Data are expressed as means ± SE (n = 5). 2B. Regression lines (data sets as presented in Graph 2A).

vasorelaxation and homeostasis. However, under pathological conditions endothelium mediates vasoconstriction by releasing vasoconstrictor substances and increasing blood pressure (Varagić, 2003). Endothelial dysfunction reduces endogenous bioactivity and identifies NO signaling as a key target for therapeutic intervention to preserve tissue integrity and minimize irreversible damage associated with hypertension and ischemic cardio-vascular disease (Milovanović et al., 2004).

The cause of spontaneous hypertension is multifactorial. A defect in the coupling of the D(1) receptor (D(1)R) to its G protein/effector complex in the renal proximal tubules plays a role in the pathogenesis of spontaneous hypertension. As there is no mutation of the D(1)R gene in SH rats, it was concluded that uncoupling of the D(1)R from its

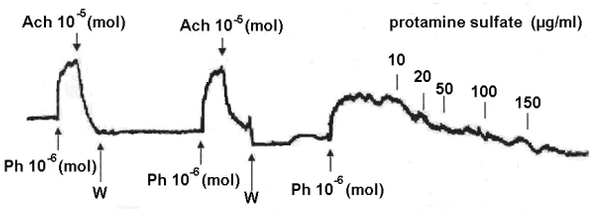


Fig. 1A. Representative recording of PS–mediated relaxation of the mesenteric artery with endothelium (E^+) isolated from normotensive rats and pre-contracted by phenylephrine (Ph). Abbreviations: Ach – acetylcholine, Ph – phenylephrine, W - washing.

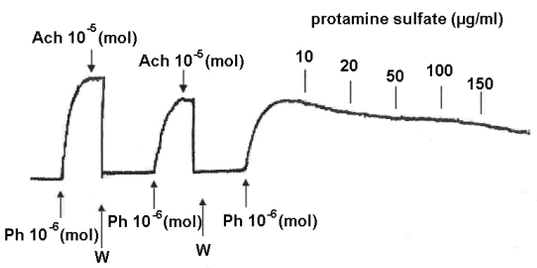


Fig. 1B. Representative recording of PS–mediated relaxation of the mesenteric artery without endothelium (E^-) isolated from normotensive rats and pre-contracted by phenylephrine (Ph).

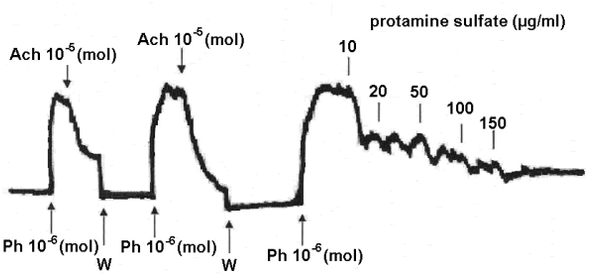


Fig. 2A. Representative recording of PS–mediated relaxation of the mesenteric artery with endothelium (E^+) isolated from SH rats and pre-contracted by phenylephrine (Ph).

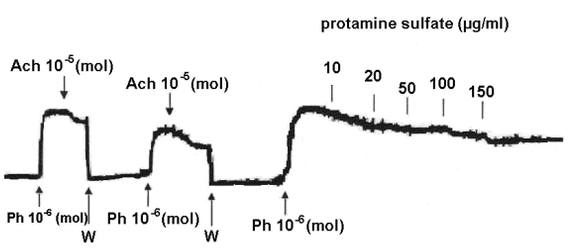


Fig. 2B. Representative recording of PS–mediated relaxation of the mesenteric artery without endothelium (E^-) isolated from SH rats and pre-contracted by phenylephrine (Ph).

G protein/effector complex in the renal proximal tubules of such rats is caused, in part, by increased D(1)R serine phosphorylation (Yu et al., 2006).

Protamine is rich in the basic amino acid arginine, which is the precursor of endothelial cell synthesis of nitric oxide. Nitric oxide is the active component of EDRF. It was shown on canine coronary, femoral, and renal arteries that protamine induced concentration-dependent relaxation in all arterial segments with endothelium that was significantly greater than in segments without endothelium (Pearson et al., 1992). The endothelium-dependent relaxation induced by protamine was inhibited by N^G-monomethyl-L-arginine (L-NMMA). L-NMMA had no effect on isolated mesenteric rings without endothelium, demonstrating that protamine stimulates the release of EDRF from arterial endothelium, and that endothelium-dependent vasodilatation may be an important cause of systemic hypotension during protamine infusion (Pearson et al., 1992).

The precise mechanism of the systemic hypotension frequently observed with the use of protamine is unclear. Although Pevni et al. (2000) reported that PS induces NO-dependent relaxation of the internal thoracic artery by activation of the endothelial nitric oxide synthase (eNOS) pathway, Takakura et al. (2006) showed on the model of isolated endothelium-denuded rat thoracic aortas that protamine and the heparin-protamine complex stimulated the release of NO from iNOS. As iNOS is induced during CPB, protamine or a heparin-protamine complex might cause systemic hypotension, at least in part, by stimulating iNOS.

Our previous results indicated that besides the difference in the function of endothelium concerned with basal $NO^\bullet$ production in normotensive and SH rats, there is a hypertension-induced difference in the smooth muscle with respect to $NO^\bullet$ relaxation (Milovanović et al., 2004). Other studies (Orešćanin et al., 2007) indicated that *in vitro* mesenteric arterial rings isolated from SH rats show attenuated relaxation in response to sodium nitroprusside (SNP) compared to rings from normotensive rats, suggesting that the total functional capacity of vascular smooth muscle to relax to nitrovasodila-

tators is altered with hypertension.

Our results indicate that besides vascular endothelium, which is the main site of PS-caused relaxation realized through release EDRF, the vascular smooth muscle also plays a significant role in PS-mediated relaxation. There are several possible mechanisms that could cause the relaxation of smooth muscle via the action of different types of receptors and signaling processes. They could involve K^+ channels, a Ca^{2+}-mediated effect, or several other receptor-mediated processes, but to date there are no data of this kind available. Recent results of ours (unpublished) obtained on isolated rat uteri indicate a role for BK_{Ca} channels in PS-mediated relaxation. This implies that hypertension might cause changes at the level of BK_{Ca} channels in the smooth muscles of blood vessels, which has to be investigated.

Acknowledgments — This work was supported by a grant from the Ministry of Science of the Republic of Serbia (project No. 143034B: "The Role of Redox-Active Substances in the Maintenance of Homeostasis").

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ЕФЕКАТ ПРОТАМИН СУЛФАТА НА ИЗОЛОВАНЕ МЕЗЕНТЕРИЧНЕ АРТЕРИЈЕ НОРМОТЕНЗИВНИХ И СПОНТАНО ХИПЕРТЕНЗИВНИХ ПАЦОВА

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Испитиван је релаксантни ефекат растућих количина (10, 20, 50, 100 и 150 μg/ml) протамин сулфата (PS) на изолованим мезентеричним артеријама нормотензивних и спонтано хипертензивних (SH) пацова са и без ендотела. PS је узроковао концентрацијски зависну релаксацију изолованих мезентеричних артерија код оба типа пацова. Како је релаксантни ефекат био слабији код SH пацова у поређењу са нормотензивним, наши резултати указују да поред васкуларног ендотела, глатки мишићи крвних судова имају веома значајну улогу у PS-посредованој релаксацији.